amyloidogenesis leads to an aggregation free energy landscape. We define the roles of and propose a classification scheme for different oligomeric species based on their location on the aggregation free energy landscape. We relate the different types of oligomers to the amyloid cascade hypothesis and the toxic oligomer hypothesis for amyloid-related diseases. We discuss existing kinetic mechanisms in terms of the different types of oligomers. We provide a possible resolution to the toxic oligomer-amyloid coincidence.

2189-Plat
Amyloid-Like Cross-Beta Structure Polymorphism: An Energetic Point of View
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Amyloid fibrils are highly ordered protein aggregates involved in numerous pathological conditions, including neurodegenerative diseases. A single mature unbranched fibril is formed from at least several interacting protofilaments, which share a common structural feature - a cross-β spine, in which β-sheets are aligned with the fibril's main axis. It has been observed that amyloid fibrils may exist with different morphologies and twists depending on their mode of preparation, even within a single sample. However, the precise etiology and pathological implications of such twist-polymorphism are unclear. We present here the results of a series of molecular dynamics (MD) simulations of a protofilament model formed by 40 copies of the GNNQQNY peptide fragment of the yeast prion protein, Sup 35. The planar protofilament observed in the crystal structure displays no free energy barrier against twisting in the absence of crystal packing interactions. Umbrella sampling simulations, in which the twist between consecutive peptides is used to control the overall protofilament's twist, confirm that the free energy minimum is observed at a 7.5 degree left-handed twist conformation. There is little apparent free energy penalty derived from twisting the cross-β structure in the range of −12 to 0 degrees. Moreover, the twist of the cross-β structure is enthalpy-driven, and while the backbone favors the straight form of the protofilament, side chains favor the twisted form. We propose that the twist of a protofilament might easily adapt to external stresses such as interactions with other protofilaments. This hypothesis is further illustrated by our characterization of different morphologies of protofilament assemblies composed of one to four protofilaments. Taken together the data support an energetic basis for the different twist-morphology states observed in amyloid fibrils.

2190-Plat
Dissecting the Membrane Dynamics of Amyloid Oligomers at a Single Molecule Level
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Fibrillar deposits of proteins are the hallmark of amyloid diseases, amongst which Alzheimer's disease stands out as the most widespread neurodegenerative pathology of the brain. Neuronal dysfunction is currently attributed to the interaction of A-beta oligomers with the plasma membrane. Several scenarios have been proposed, but the mechanisms of binding of the oligomers to the cell membrane and their subsequent toxicity is still unclear. Distinct results indicate that oligomers may insert non-specifically into the lipid bilayer, or bind to specific targets, such as post-synaptic structures or gangliosides characteristic of lipid rafts. In general, these studies have investigated the averaged features of an ensemble of molecules.
Here, we have been able to successfully monitor the mobility of single A-beta oligomers on the plasmamembrane of living neuroblastoma cells. Preformed oligomers were incubated with cells and subsequently labelled with monoclonal primary antibodies and secondary Fab fragments coupled to quantum dots (QDs). Single QDs bound to the oligomers were then tracked.
The analysis of the trajectories reveals that most of the oligomers show a highly confined membrane mobility, suggesting a potential involvement of the cytoskeleton, while some diffuse laterally following a free Brownian motion. Strikingly, we found that other amyloid aggregates sharing a similar conformational structure but composed of different proteins (amylin and prion Sup35) display comparable dynamics. Moreover, we discovered that the presence of amyloid aggregates decreases dramatically the membrane diffusion of GM1 gangliosides labelled with biotinilated cholera toxin coupled to streptavidin-QDs.
Overall, these results enable a better understanding of the basic mechanisms underlying Alzheimer's diseases and other amyloid pathologies.

2191-Plat
Physical Properties of Yeast Prion Proteins Studied with Optical Tweezers
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Formation of amyloid fibers plays a vital role in both natural biological processes and neurodegenerative disease. Recently, amyloid formation has been shown to be a general property of proteins and peptides. Their impressive mechanical properties, which are comparable to spider silk, combined with their ease of assembly in synthetic preparations make amyloid fibers particularly suited for nanomaterials applications, including as templates for conducting nanowire formation, as scaffolds for cell growth, and as functionalized biosensors. Prion proteins are a special class of amyloid fiber forming proteins which are self-templating and thereby transmissible as disease vectors. This work combines optical tweezers force spectroscopy with fluorescence imaging to study the physical properties of amyloid fibers formed from polymorphic variants of a 253 amino acid N-terminal fragment (NM) of the yeast prion protein Sup35. Experiments revealed that fibers associated with a ''weak'' NM prion strain have an approximately 2-fold larger bending stiffness than those associated with a ''strong'' NM prion strain. We further subjected NM fibers to multiple cycles of forces up to 250 pN resulting in unfolding of individual prion subdomains and rupture of intermolecular interactions. Our results have implications for the physical basis of prion strain diversity and give important insights into the underlying structure of Sup35 prions.

2192-Plat
Probing the Conformational Ensemble of Polyglutamine During the Initial Stages of Aggregation
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Nine different neurodegenerative diseases, including Huntington's disease, are associated with the aggregation of proteins whose only commonality is a repeating stretch of glutamine. Experiments and computer simulations have demonstrated that monomeric forms of polyglutamine molecules sample heterogeneous sets of collapsed structures in water. Molecular simulations have predicted that these molecules spontaneously associate at conditions approaching those of typical in vitro experiments for chains of length N>15. Moreover, the spontaneity of these homotypic associations increases with increasing chain length. These results suggest that polyglutamine aggregation is unlikely to follow a homogeneous nucleation mechanism, which is currently the most widely accepted mechanism by which polyglutamine aggregation is thought to occur. In this work, we test these predictions using both steady state and time resolved Förster Resonance Energy Transfer (FRET). Hopefully this work, along with the simulation results, will allow a better understanding of how monomeric polyglutamine assembles into soluble oligomers and, eventually, insoluble aggregates.

2193-Plat
Changing the Kinetics of Amyloid Beta Plaques Formation: Implications for Alzheimer's Disease Immunotherapy
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Clear evidence exists linking the presence of neuritic amyloid beta (Aß) peptides plaques with the brain's tissue deterioration and cognitive impairment in patients with Alzheimer's disease (AD). Removing these plaques results a logic approach to treat patients with AD. Understanding the plaque formation mechanisms is key to developing strategies to remove them. In previous studies our group has investigated the plaque formation process using attenuated total reflection Fourier infrared (ATR-FTIR) spectroscopy and atomic force microscopy (AFM), observing how different Aß peptide aggregates formed and under what kinetic conditions they assembling into mature fibrils. We have been able to determine the changes in the secondary structure of the peptide molecules during this process. In this work, we combined the same analytical techniques to investigate the use of different anti-Aß monoclonal antibodies to analyze the process of destabilization and prevention of AD plaque formation. We compared the changes of kinetic rates of fibrillization when the peptides were incubated with different antibodies from the early stage of aggregation, at pH 7.4 and 37°C. The molar ratio of antibodies to peptide used was 1:1000. We found that some antibodies considerable decrease the formation of parallel beta sheets structures increasing the formation of alpha helix structures or unordered,

driving the peptide away from normal fibrillization and rather producing amorphous aggregates. The results of the peptide's morphological evolution and secondary structure changes revealed that some antibodies have the potential of preventing formation of parallel beta sheets structures, which may be an indication of Aß fibrillization preclusion. This work provides insights to understand the mechanistic effects in which antibodies alter the secondary structure of amyloid peptides modifying their fibrillization, critical for Aß plaque clearance in the brain of patients with AD.

Platform AK: Membrane Structure II

2194-Plat

Protein-Lipid Interactions are Determinants of Small and Large-Scale Membrane Domains

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Sphingolipids and cholesterol exhibit preferential association that cells employ to compartmentalize their membranes and regulate protein function. Separation of artificial membranes into liquid-ordered (Lo) and liquid-disordered (Ld) phases is regarded as a common model for this compartmentalization. However, tight lipid packing seems to conflict with efficient partitioning of transmembrane (TM) proteins into the Lo phase. To assess membrane order as a component this organization we performed fluorescence microscopy with the membrane probe C-laurdan. We compared Lo-Ld phase separated model membranes with plasma membrane systems that exhibit inducible phase separation: giant plasma membrane vesicles (GPMVs) and plasma membrane spheres (PMS). Notably, only the latter support selective inclusion of TM proteins with the sphingolipid GM1 into one phase. We found comparable small differences in order between the separated phases of both biomembranes. Lateral packing in the ordered phase of GPMVs resembled the Lo domain of model membranes, whereas the GM1 phase in PMS exhibited considerably lower order suggesting that cholesterol-mediated tight lipid packing is insufficient to explain the coalescence of a TM protein-selective lipid phase as seen in the PMS. To further investigate basic interactions between TM proteins and lipids that might contribute to protein-selective phase behavior we analyzed the order of model membranes into which we integrated a TM peptide. We found that the peptide affected the order of bilayers in a cholesterol and acyl chain length-dependent manner. The results show that TM proteins influence their local lipid environment and thus have the potential to become determinants in the formation of membrane domains.

2195-Plat

N-3 Polyunsaturated Fatty Acid Incorporates Directly into Lipid Rafts to Disrupt Domain Clustering and MHC Lateral Organization of Antigen Presenting Cells

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N-3 polyunsaturated fatty acids (PUFA) are under clinical testing for the treatment of symptoms associated with inflammatory disorders such as cardiovascular disease. However, effective use n-3 PUFAs as nutraceuticals has been limited by a poor understanding of their molecular mechanisms. Here we addressed how the n-3 PUFAs eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids modified lipid raft and protein lateral organization of antigen presenting cells, whose function is suppressed by n-3 PUFAs. Quantitative fluorescence microscopy showed that DHA, but not EPA, relative to controls, diminished lipid raft clustering and increased their size. A significant amount of DHA incorporated directly into rafts without changing cholesterol distribution between rafts and non-rafts. Quantification of fluorescence co-localization images showed that DHA selectively altered the lateral organization of the major histocompatibility complex (MHC) class I protein. FRET microscopy measurements showed that DHA modified MHC class I clustering on a nanometer scale. Taken together, our findings are not in agreement with studies in model membranes on unsaturated fatty acids and lipid rafts. Therefore, we propose a new model, which reconciles contradictory viewpoints from biophysical and cellular studies, to explain how an unsaturated fatty acid modifies lipid rafts on several length scales. Our study provides mechanistic details by which DHA suppresses antigen presenting cell function, which allows us to more effectively use these fatty acids in the clinic.

2196-Plat

Detection of Domain Formation in a Subpopulation of Late Endosomes by Templated J-Aggregates

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Although controlling the self-assembly of molecules to form specific structural motifs is important for the design of materials with unique optical or electronic properties, the growth of these assemblies also provides useful information regarding their local environment. We have previously reported the formation of templated J-aggregates of an organic chromophore on model supported planar bilayers [Langmuir, 25, 10719], finding that the spectral characteristics of the J-aggregates reflected the structure of the nucleating lipid bilayer. Here, we describe how the structure of membrane domains on internal organelles in live cells can be inferred from the presence of specific J-aggregate forms. Cell lines expressing GFP-conjugated wild-type Rab5a or Rab7, GTPase markers of early and late endosomes, respectively, were treated with dyes and studied using confocal microscopy and fluorescence spectroscopy. Remarkably, while we did not observe any significant co-localization of the GFP-Rab5a marker and J-aggregates, there was clear evidence that the J-aggregates were present within late endosomes labeled with GFP-Rab7. Close inspection revealed that the J-aggregates were confined to smaller vesicles within the lumen of the late endosome. These in vitro results were compared with J-aggregates formed on multi-component planar phospholipid bilayers mimicking the lipid compositions of late endosomal and mitochondria. Correlated confocal fluorescence and atomic force microscopy revealed that a lipid enriched only in late endosomes was responsible for the formation of these J-aggregates. Live cell imaging suggested that these structures were present during an early phase of late endosome maturation, after Rab5 conversion but prior to acidification.

2197-Plat

Micropatterning of Plasma Membrane Proteins to Analyze Raft Localization in Living Cells

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We have developed an assay for quantitative analysis of the interaction between a fluorescently marked protein (prey) and a membrane protein (bait) using microstructured surfaces covered with biotinylated ligands (antibodies) targeted against the bait. The proof-of-concept was demonstrated for the interaction between CD4, a major co-receptor in T-cell signalling, and Lck, a protein tyrosine kinase essential for early T cell signalling. Here we present improvements and a more precise characterization of the method as well as the applicability of the assay for the analysis of protein interactions within lipid rafts in the inner and outer leaflet of the plasma membrane. We stably expressed fluorescently labelled raft and non-raft proteins in the human T24 cell line as prey proteins and determined the degree of interaction with the antibody-targeted bait proteins CD59 (GPI-anchored protein, raft marker) and CD71 (Transferrin-receptor, non-raft marker), respectively. We found strong interaction of CD59 with putative raft markers including various GPI-GFP constructs, the inner-leaflet associated proteins Lck and Flottilin1 and a Pleckstrin-Homology domain fused to GFP. Importantly, we did not find interaction of CD59 with CD71-GFP and other potential non-raft proteins. When CD71 was used as the bait protein we did not find interaction with the putative raft markers. While the detected absence of CD71 from and the presence of CD59 in lipid rafts confirm current knowledge, it is still very unclear if a lipid-raft dependent coupling of proteins and certain especially negatively charged lipids across the plasma-membrane bilayer exists. Thus, our micropatterning assay will be of great interest to address this question.

2198-Plat

Differentiating Lipid Phase Domains in Cells using Fluorescence Lifetime of DiI

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Dynamic heterogeneous distribution of lipid phases is thought to be an important means by which lipids modulate cellular biology. However, information about these domains is not accessible using conventional optical microscopy because their size (10-100nm) is well below the diffraction limit. Fluorescence lifetime (FL) of variable chain length di-alkyl carbocyanine dyes (DiIs) has been proposed to reflect lipid phase, but verification that fluorescence readouts reflect lipid phase in cells is lacking. Thus, we used time-correlated single photon counting techniques to test the fidelity with which photophysical properties of DiI-C12 and DiI-C18 report lipid order in cells. DiI FL increases with increased solution viscosity (1 to 70 cP) were independent of acyl chain length, demonstrating that lifetime reports chromophore headgroup local viscosity. Sensitivity of FL of DiI to membrane order was evaluated in giant unilamellar vesicles (GUVs) composed of DOPC (liquid-disordered), DOPC:Chol (liquid ordered), DPPC:Chol (liquid ordered), and DPPC (gel phase). FL of DiI increased significantly with increase in membrane order, and correlated well with a decrease in diffusion coefficient. Phase partitioning of DiI-C12 and